Veterinary Integrative Science 2019; 17(1): 1-10

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

# Lung consolidations assessment and associated bacterial pathogens detection in slaughter-aged pigs in Chiang Mai - Lamphun, Thailand

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## Abstract

Lung scoring assessment for consolidated lesions at the slaughtering level can be measured the severity of bacterial respiratory problem occurring at farm level. This study aims to evaluate the extent of lesion and identify the associated bacterial pathogens in slaughter-aged pigs using lung scoring and bacterial culture with PCR confirmation, respectively. The results obtained in this study could be applied in pig respiratory disease controls throughout the fattening period. From September 2016 to December 2016, a cross-sectional study was performed in 646 lung samples from 17 farm batches conducted across 3 slaughterhouses in Chiang Mai and Lamphun, Thailand. Three hundred and seventy-three (57.74%) of lung samples showed consolidation. The mean lung score for all lungs and consolidated lungs were 8.78 and 15.21, respectively. Forty-six randomly selected lung samples were tested for bacteria identification. Twenty, eleven and 13 samples tested positive for *M. hyopneumoniae*, *M. hyorhinis* and *P. multocida* type A, respectively. Seven lung samples had mixed infection. There was no significant difference between bacterial pathogen detected by PCR results and lung lesion score ($p>0.05$). This study concluded that bacterial pathogen-related pig respiratory problems are an important issue that warrants further study. Moreover, the bacterial pathogens identified from the affected lungs and vaccinated bacterins-type widely use in the area, were analogous. Strategies for bacterial pathogen control other than vaccination should be explored.

**Keywords:** Pig, Lung consolidation, Lung scoring, Slaughterhouse

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***Article history:*** *received manuscript: 7 June 2018, revised manuscript: 16 July 2018, accepted manuscript: 15 August 2018, published online: 14 September 2018*

***Academic editor:*** *Korakot Nganvongpanit*

# INTRODUCTION

Porcine respiratory diseases are a significant problem that can cause economic losses in intensive pig production systems worldwide (Maes et al., 2008; Palzer et al., 2008). Bacterial infection is considered as a major cause of respiratory infections in pigs (Došen et al., 2007). During the fattening period, the problem could not be seen as easily (Sibila et al., 2007). The issue is often overlooked as it is associated with low mortality and morbidity rates. However, it may result in reduced growth performance and feed conversion efficacy. Moreover, secondary infection from other pathogens, especially porcine reproductive and respiratory syndrome virus (PRRSv) playing in the porcine respiratory disease complex (PRDC) is linked (Grest et al., 1997; Martelli et al., 2006).

Lung consolidation (*Mycoplasma hyopneumoniae*-like gross pulmonary lesion) are the most commonly correlated with bacterial pneumonia. *M. hyopneumoniae* is recognized as the primary etiological bacterial pathogens of respiratory disease (Otagiri et al., 2005). However, *M. hyosynoviae*, *M. hyorhinis* and *Pasteurella multocida* are also correlated with changes in abnormalities (Christensen and Mousing 1999; Došen et al., 2007). Consolidated lungs consist of strength solid texture, and are mainly located bilaterally in the cranioventral regions of the affected lung. The lesions present in two different colours, depending on the duration of infection: red (red hepatization) is caused by red blood cells and accumulation of fibrous and inflammatory cells in the lung, while grey (grey hepatization) is caused by the breakdown of the accumulated red blood cells (Maes et al., 2008). To quantify a lesion, scoring at the postmortem examination is necessary to assess the extent of pathological changes. The method is based on the visual evaluation and palpation of lungs. In such a scenario, retrospective evaluation of the lesion from lungs at slaughter (lung scoring) can be used to determine the incidence or severity of respiratory problems during the fattening period (Morante et al., 2015). This approach is accepted as one of the important tools used to examine infections in herds (Fraile et al., 2010; Straw et al., 1989; Van Staaveren et al., 2016).

In Northern Thailand, lung scoring surveillance data is non-existent. Subclinical respiratory disorders go unnoticed in some herds. The actual conditions in farms are concealed; consequently, proper herd management cannot be carried out (Sibila et al., 2007). Accordingly, the present cross-sectional study conducted between September 2016 and December 2016 aimed to assess consolidated lung lesion scores, and to identify the bacterial pathogens associated with this parameter among slaughter-aged pigs in Chiang Mai and Lamphun. Acquiring information regarding pig respiratory disorders could help improve the growth performance throughout the fattening period in the study area.

# MATERIALS and METHODS

## Sample size calculation

Chiang Mai and Lamphun provinces are known as area with the highest densities of intensive pig farming in northern Thailand, were selected as the study areas. Using “Epi Tools - Sample size calculations: to estimate a single

proportion" from http://epitools.ausvet.com.au/ content.php ?page=Sample-Size, it was determined that at least 601 samples were required. The estimated truth proportion was 50%. Also, 0.95 and 0.04 were selected as the confidence level and desired precision parameters, respectively.

## Lung scoring examination

From September 2016 to December 2016, 646 lung samples were obtained from 17 farm batches across 3 slaughterhouses. A batch was defined as group of fattening pigs belonging to the same farm, slaughtered on the same day in each slaughterhouse. All lungs were evaluated for the extent of consolidated lesion. It was assessed as described previously (Straw et al., 1989). The method is considered separately 7 pulmonary lobes examination. The extent of lung consolidation was represented as percentage per lung. This outcome of each affected lobe was multiplied by its relative size. The maximum scores for each lung were as follows: twenty for apicals (10 × 2), twenty for cardiacs (10 × 2), fifty for diaphragmatics (25 × 2), and ten for an intermediate (10), with a total possible score of 100 (Figure 1). Finally, the individual lung lesion score and the average lung lesion score within each farm batch were recorded by 2 veterinarians.

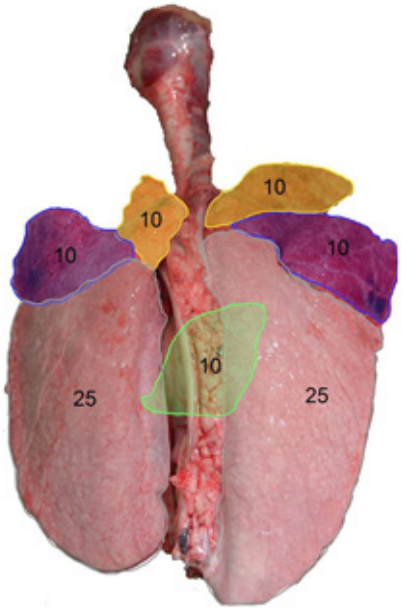


**Figure 1** Summary of Straw et al., (1989) lung scoring systems. The percentage of lesion in each lobe area is related to size and summed to provide the total area percentage.

### Bacterial pathogen identification by bacterial cultivation and polymerase chain reaction (PCR)

Consolidated lesions (only red hepatisation) were collected from the 46 randomly selected lung samples. All of them were shipped in an icebox within 24 hours of collection to the Section of Bacteriology, Veterinary Research and Development Center (Upper Northern Region), Lampang, Thailand, for bacterial pathogen identification. The samples were inoculated on blood agar (Oxoid; Cambridge, UK) supplemented with 5% defibrinated sheep blood and on MacConkey agar (Oxoid) at 37ºC overnight. Brain-heart infusion broth (Oxoid) was used for the propagation and maintenance of bacterial cultures (Gilsson, 2008). The materials taken were further identified by PCR technique. PCR was performed according to the method previously described (Kobayashi et al., 1996; Makhanon et al., 2012; Mattsson et al., 1995; Townsend et al., 2001).

### Statistical analysis

The prevalence of consolidated lung, individual lung lesion score and average lung lesion score within farm batches were analyzed for descriptive statistical analysis by open source statistics for public health from http://www.openepi.com. Additionally, Kruskal-Wallis test used to compare lung lesion scores between the bacterial pathogen identification results was performed by R-studio®. The P value lower than 0.05 is remarked as statistical significantly difference.

## RESULTS

Six hundred and forty-six lungs tested, two hundred and seventy-three healthy lungs were demonstrated (42.26%; 95% CI: 38.51-46.10). Remains of 373 lungs had cranio-ventral pulmonary lung consolidation (57.74%; 95% CI: 53.90-61.49). Additionally, average lung lesion score concerning only consolidated lungs was 15.21 (95% CI: 13.68-16.74) whereas average lung lesion score in all lungs tested was 8.78 (95% CI: 7.73-9.84).

Considering in only consolidated lung, most lung scores were placed in the range with 1-10 (183 lungs; 28.32%; 95%CI: 24.99-31.92). The individual consolidates were detected in all farm batches. In addition, the most frequently of the lung lesion scores average detection within-farm batch was ranged in 1-10 (12 farm batches; 70.59%; 95%CI: 46.87-86.72) (Figure 2).

Forty-six randomly selected consolidated lungs, twenty (43.47%; 95% CI: 30.21-57.75), eleven (23.91%; 95% CI: 13.91-37.93) and thirteen (28.26%; 95% CI: 17.32-42.55) samples were positive with *M. hyopneumoniae*, *M. hyorhinis* and *P. multocida* type A, respectively. For the 9 remaining samples were negative for any bacterias. Of PCR-positive results, seven lung samples had mixed infection (15.21%; 95% CI: 7.57-28.22) (Figure 3), with the lung lesion score range of 3.5- 64. Moreover, in Kruskal-Wallis analysis, there was no significant difference between PCR results and lung lesion score ($p>0.05$) (Figure 4).

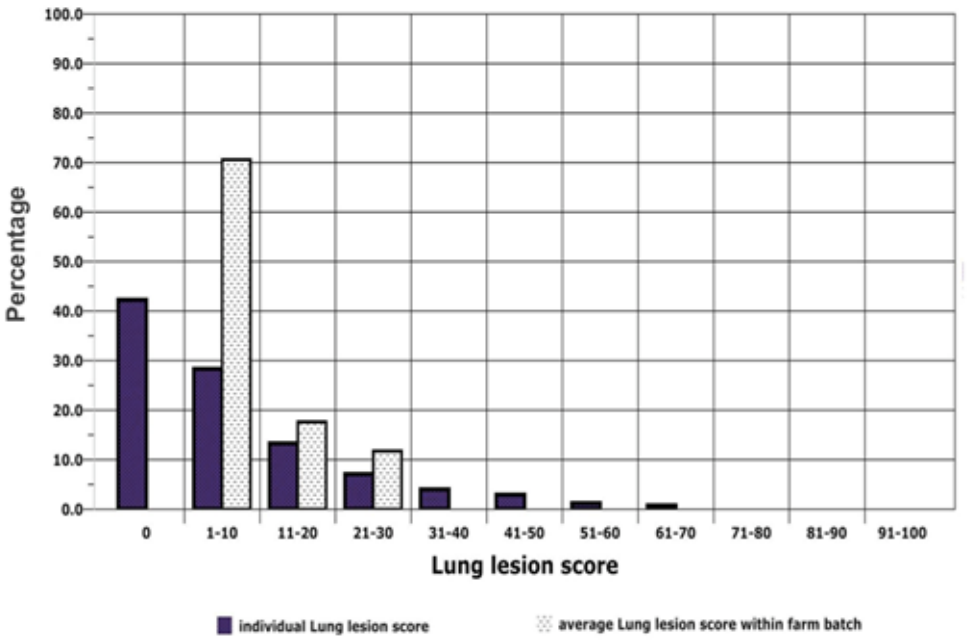


**Figure 2** Distribution of individual lung lesion score and average lung lesion score within-farm batch from 646 pigs in Chiang Mai-Lamphun provinces during September-December 2016.

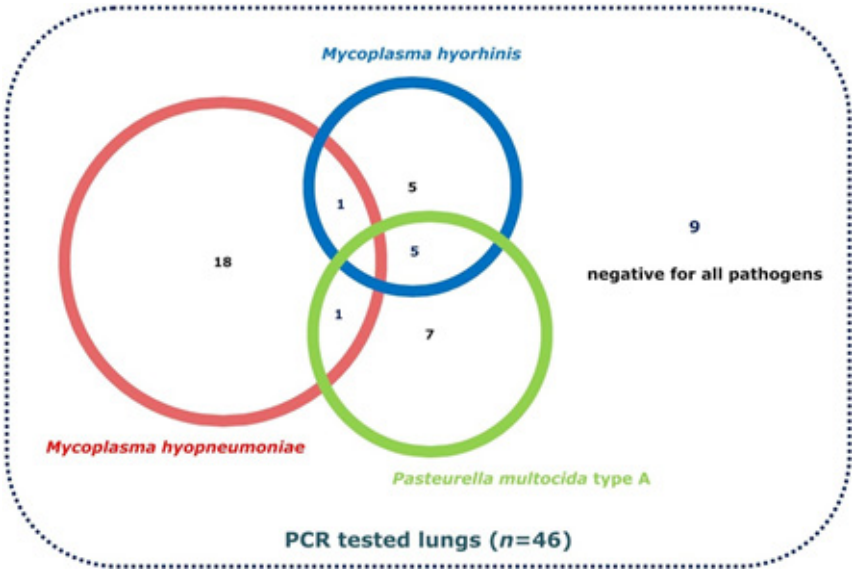


**Figure 3** Frequently distribution of pathogen identified by bacterial cultivation with PCR confirmed from 46 randomly selected lungs in Chiang Mai-Lamphun provinces during September-December 2016.

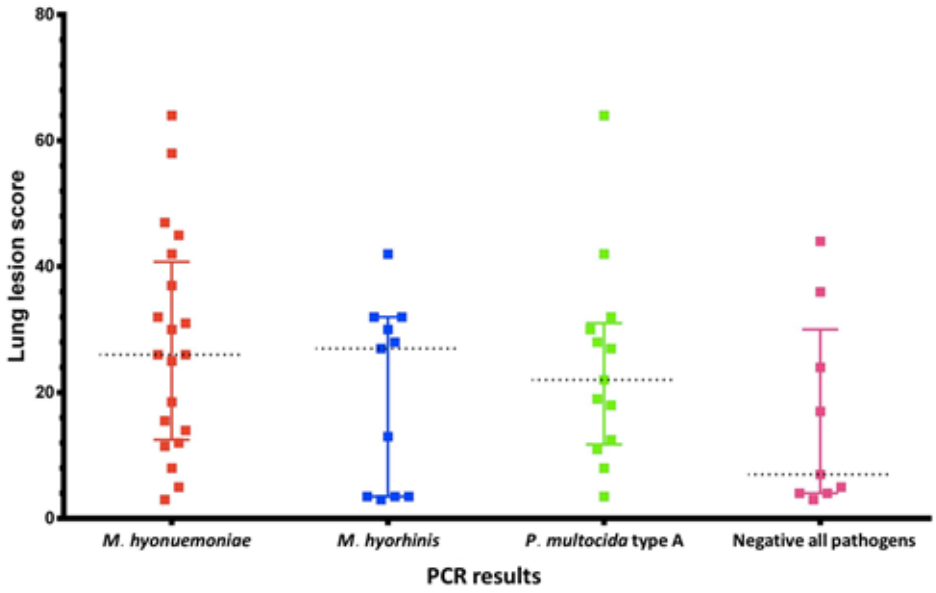


**Figure 4** Individual lung lesion score with median and interquartile range separating with pathogen identification results from 46 randomly selected lungs in Chiang Mai-Lamphun provinces during September-December 2016

## DISCUSSION

Pig respiratory disease problem could be inferred by the slaughtering inspection data. Lung consolidation lesion (*M. hyopneumoniae* like lesion) was commonly noticed from slaughter-aged pigs in Chiang Mai-Lamphun, during September-December 2016. Indicating, this lesion type was wide-spread in pig herds in the study area. Obtaining prevalence of consolidated lung was similar to the published findings of 56% in Spain (Fraile et al., 2010) and 58% in Ireland (Van Staaveren et al., 2016). However, considered in the average of lung lesion scores, the scoring system used in previously studies were not the same with method tested in current study. Recorded on the scale of 0-28 and 0-50 was done in the studies in Spain and Ireland, respectively. Therefore, severity of the lesions on the scale of 0-100 in this study could not be compared.

All farm batches testing had certain of pigs with lesion, signifying that at least a pig was infected in late production cycle and develop lesion close to slaughter-aged (Fraile et al., 2010). In addition, the lung lesion score values that were greater than 30 the individual lung were referred as severity bacterial infected condition at fattening period. They were detected approximate of 8% in current study. Most of them were belonged to 5 farm batches related with high respiratory problem (lung lesion score average within farm batches>10). The findings indicated the occurring of respiratory problem in herd level, and possibility persistent for long duration (Maes et al., 2008). Reduced in growth performance, such as average daily gain (ADG) and feed conversion rate (FCR) might be prolonged (Sibila et al., 2014).

*M. hyopneumoniae*, *M. hyorhinis* and *P. multocida* type A were identified in this study. Since the test was examined only consolidated lungs, acquiring results could not be represented for the infection prevalence of slaughter-aged pig in study area. The extent of lung lesion is not associated with type of bacterial pathogens. Indicating the virulence of *Mycoplasma* spp. and *P. multocida* type A are not difference. However, the infection from mixed bacterial pathogens was noticed. The finding was related to the previous described. Common infection from various species of *Mycoplasma* spp. could be detected that could be induced pasteurellosis for the secondary infection (Glisson, 2008; João et al., 2015; Palzer et al., 2008). Approximately of 80% of lungs tested were positive at least one bacterial pathogen. Routine practices of pig herds in the study area were not enough to protect the infection in fattening period. Progressing of the lesion had been presented in slaughter-aged. Antibiotics using is deliberated as the factor can deal with respiratory disorders (Bousquet et al., 1997). Tiamulin, tylosin, lincomycin have been effective against *Mycoplasma* spp. (Thongkamkoon et al., 2015). Meanwhile, ceftiofur, enrofloxacin and florfenicol are remained susceptible against *P. multocida* (Portis et al., 2013). However, withdrawal period at least a month before go to slaughters is needed to avoid drug residuals (Reynolds et al., 2009). For that reason, vaccination was considered as another choice solved together. Remarkably, almost a half of lung tested in current study were positive with the bacterial pathogen. Efficacy of the vaccine might be insufficient to control the disease throughout the life of fattening pigs. For vaccination schedule recommended, double doses at 3 and 5 week olds should be carried out (Stephen et al., 2013). Alternatively, two or three week olds pigs can be vaccinated with longer-lasting protection single dose bacterins, which can provide the advantage of labor saving and pigs stress reduction (Martelli et al., 2006). These vaccinated schedules are affected within the conditions of the piglets, descent from non-vaccinated sows at gestating period. In addition, *M. hyopneumoniae* vaccine was the tool for decreasing clinical signs and lung lesion (Maes et al., 2008; Martelli et al., 2006). However, it could not protect the pigs far from the infection (Sibila et al., 2007). As mentioned above, other control strategies should be implemented. The good practices for general management, such as upright biosecurity measures, all in/all out production and housing condition improving are realized. It could be enhanced the efficacy of antibiotics and vaccines, as well as decreased the occurrence of many other diseases involved (Fraile et al., 2010).

## CONCLUSION

This study was provided the first data of lung scoring assessment and the associated bacterial pathogens in Northern-Thailand. Based on the findings, pig respiratory disease problem was recognized as the one of important issue in study area. Consolidated lung with high average mean scores were investigated from most slaughter-aged pigs. Several control measures over the vaccination program, such as proper antibiotics using, farm biosecurity, as well as optimal housing conditions are recommended, to control the disease and improve growth performance throughout fattening period.

## ACKNOWLEDGEMENT

This study was supported by the funding of Bayer Thai Co., Ltd. and Faculty of Veterinary Medicine, Chiang Mai University, Thailand. We wish to thank technicians of Section of bacteriology, Veterinary Research and Development Center (Upper Northern Region), Lampang, Thailand who helped with sample processing. The authors are grateful to the slaughterhouse staffs and farm owners that collaborated by supplying with data included in the study.

## AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: PT PhT. Performed the experiments: PT PTo JT PhT. Analyzed the data: PT PP PhT. Contributed reagents/materials/analysis tools: PTh. Wrote the paper: PT PhT.

## CONFLICT of INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**How to cite this article;**

Pakpoom Tadee, Patiparn To-in, Jakkraphan Thongjamroon, Prapas Patchanee, Patcharee Thongkamkoon and Phacharaporn Tadee. Lung consolidations assessment and associated bacterial pathogens detection in slaughter-aged pigs in Chiang Mai - Lamphun, Thailand. Veterinary Integrative Sciences. 2019; 17(1): 1-10